

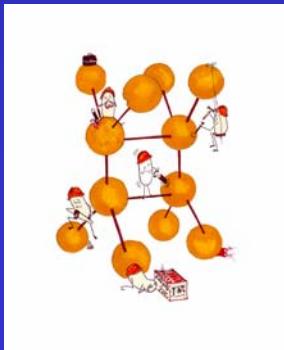


**All rights reserved. No part of this presentation  
may be reproduced or transmitted, in any form  
or by any means, without expressed permission  
of the author.**

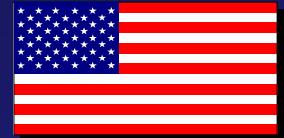


**B. Thomas Johnson, PhD**  
**Environmental Microbiology**  
**Columbia Environmental Research Center**  
**Columbia, MO USA**

*Email: [btjohnson@usgs.gov](mailto:btjohnson@usgs.gov)*



B.Thomas Johnson, CERC-USGS



**USGS and Department of Defense Environmental  
Program Conference  
Biloxi, MS**

**Monitoring toxicological changes  
in sediments during  
biotransformations of munitions :  
microbial community profiling**

**B. Thomas Johnson<sup>1</sup>, M. Nipper<sup>2</sup>, and S. Carr<sup>1</sup>**  
**Columbia Environmental Research Center<sup>1</sup>, USGS, Columbia, MO**  
**USA, Texas A & M University <sup>2</sup>at Corpus Christi, TX USA**

**3-7 May 2004**



# A Paradigm Shift:

## Metabolic Fingerprint of Environmental Bacteria

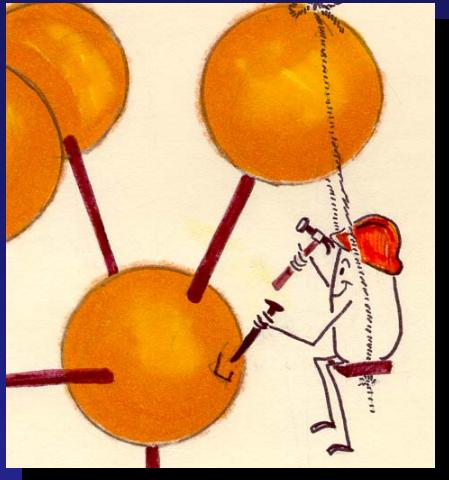
B.Thomas Johnson, CERC-USGS



# The Search for Aquatic Pollutants

## The Indicator Paradigm

**Indicator microorganisms are used globally as a warning device of possible microbial and chemical contamination and as indices of aquatic toxicity, quality and health concerns.**



## The Bacterial Indicator Paradigm:

- ✓ **Bacteria produce enzymes**
- ✓ **Different bacteria produce different enzymes**
- ✓ **Different enzymes are inhibited by different toxins**
- ✓ **Enzyme specific tests identify specific bacteria**



# Aquatic Risk Assessment



- The Issue
  - Risk of water-borne environmental chemopollutants and biopollutants
- The Problem
  - Ecological and toxicological
- The Approach
  - Detection with bacterial indicator organisms
- The Method
  - Enzyme-Specific Tests

# Uses of Indicator Bacteria in Aquatic Risk Assessment



- Eutrophication
- Toxicity
- Perturbation
- Biodiversity

# Toxicological Risk Assessment: Water

Indicator  
Bacteria

The Search for  
Microbiological  
Perturbation



B.Thomas Johnson, CERC-USGS

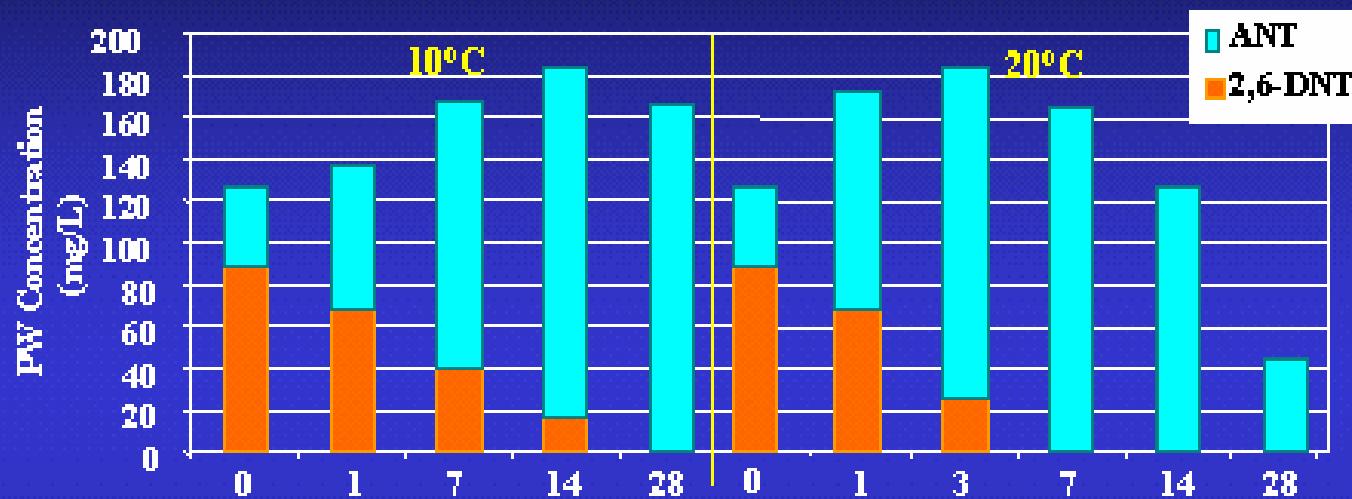
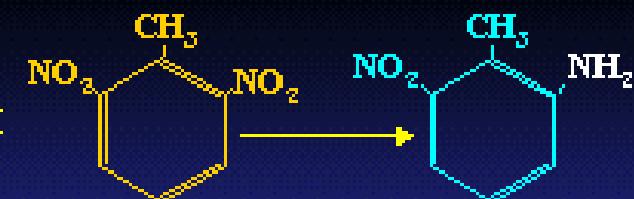
The background image shows a wide expanse of wetland or marsh area. In the foreground, there's a field of tall, green grass. Beyond the grass, the land is covered in shallow pools of water, creating a pattern of light-colored patches. In the distance, across the water, there's a line of trees and possibly some buildings. The sky above is filled with wispy, white clouds.

# Total Heterotrophic Bacterial Activity:

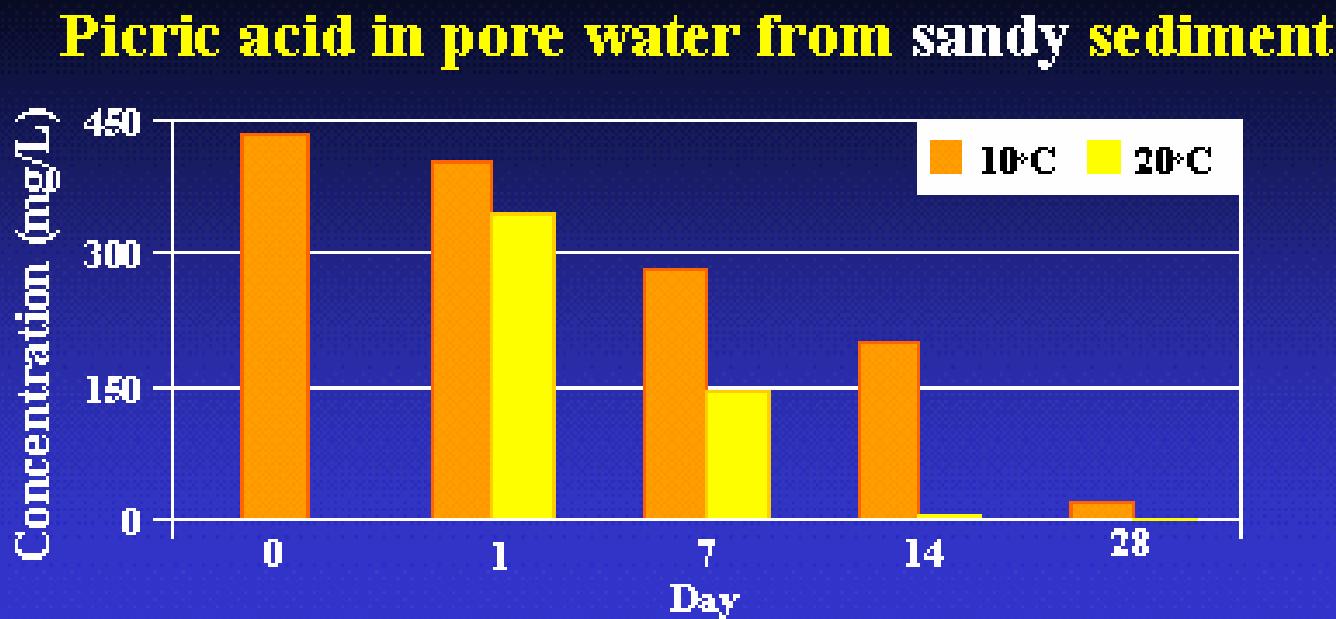
- Microbial Perturbations
- Measurement Microbial Biodiversity

# Texas Control (LAR) Sediment: 2,6-DNT Degradation

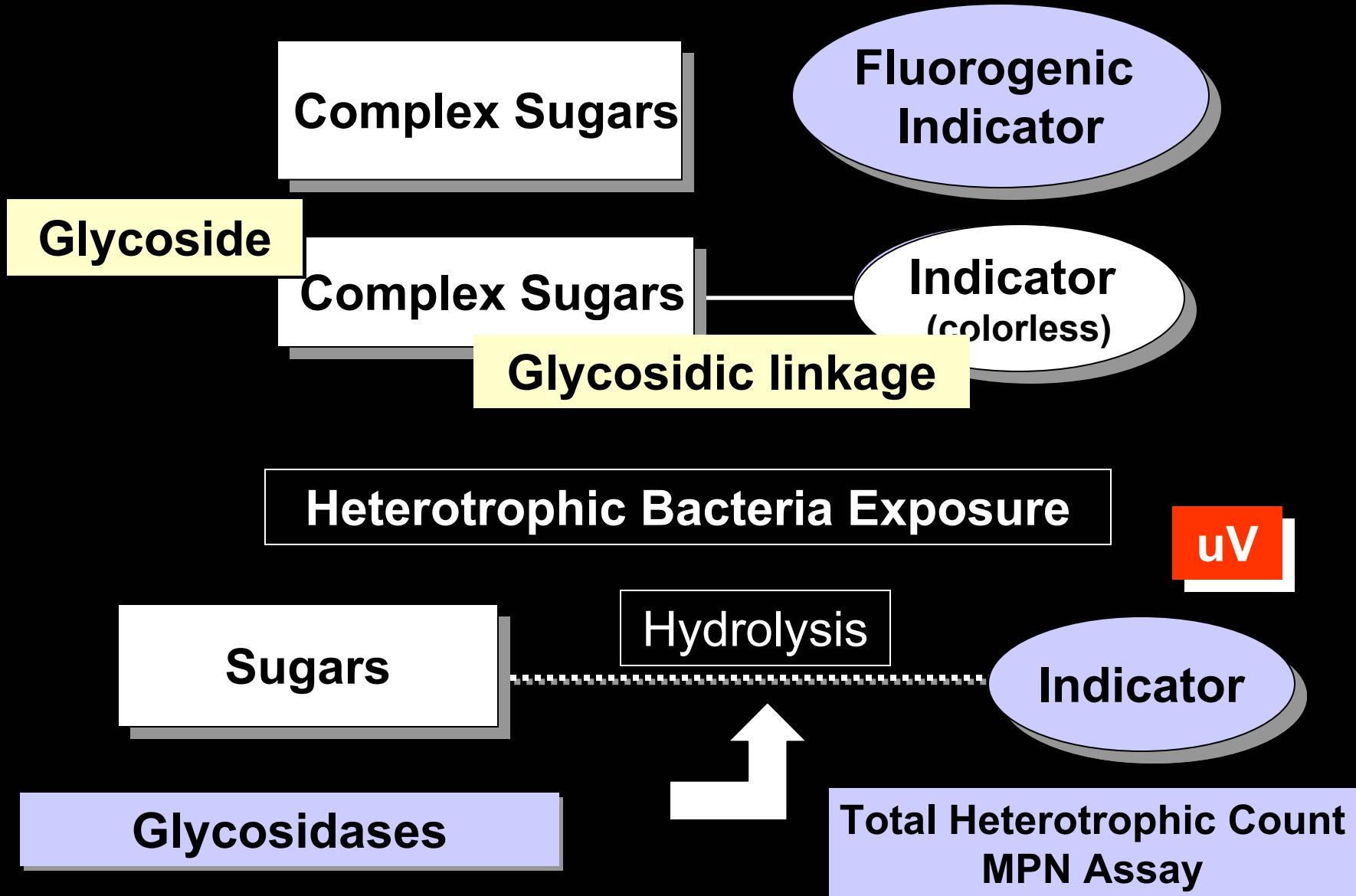
2,6-DNT and 2-amino-6-nitrotoluene  
(2-methyl-3-nitroaniline) in sediment  
and pore water from sandy sediment



# Texas Control (LAR) Sediment: Picric Acid Degradation



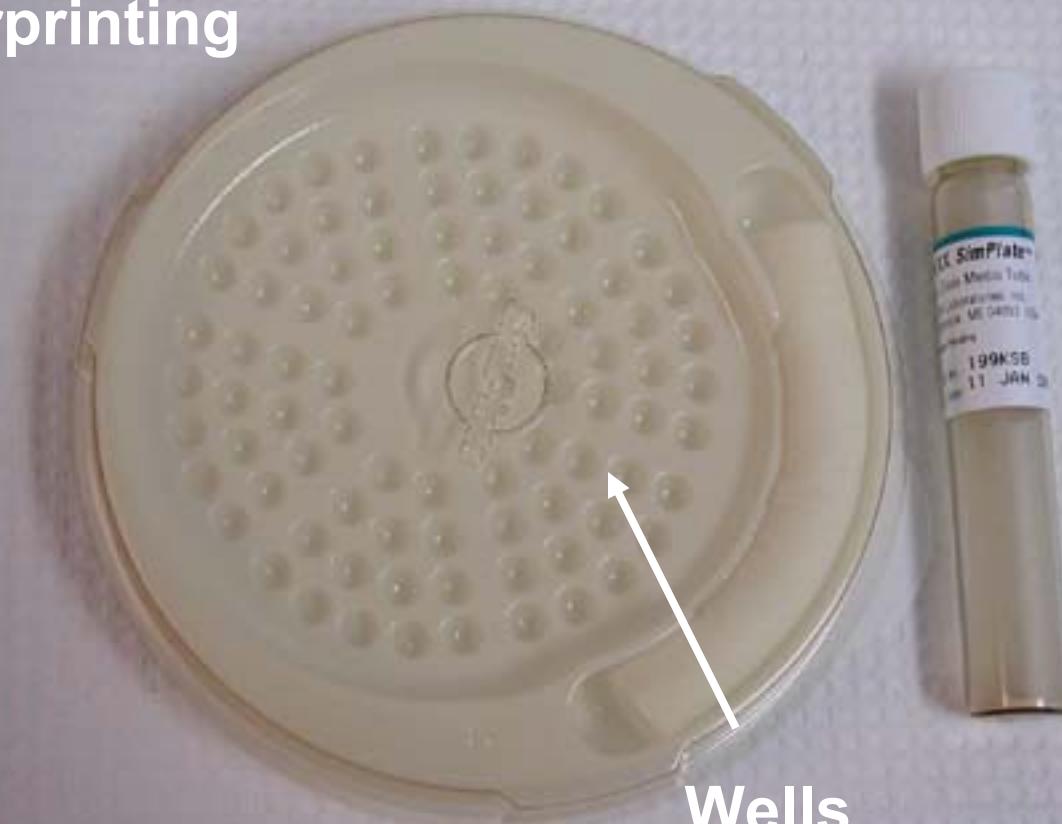
# Defined Substrate : Enzyme Specific Tests - THC



# Munitions Degradation Study: Puget Sound Sediment Analysis: Total Heterotroph Bacterial Count

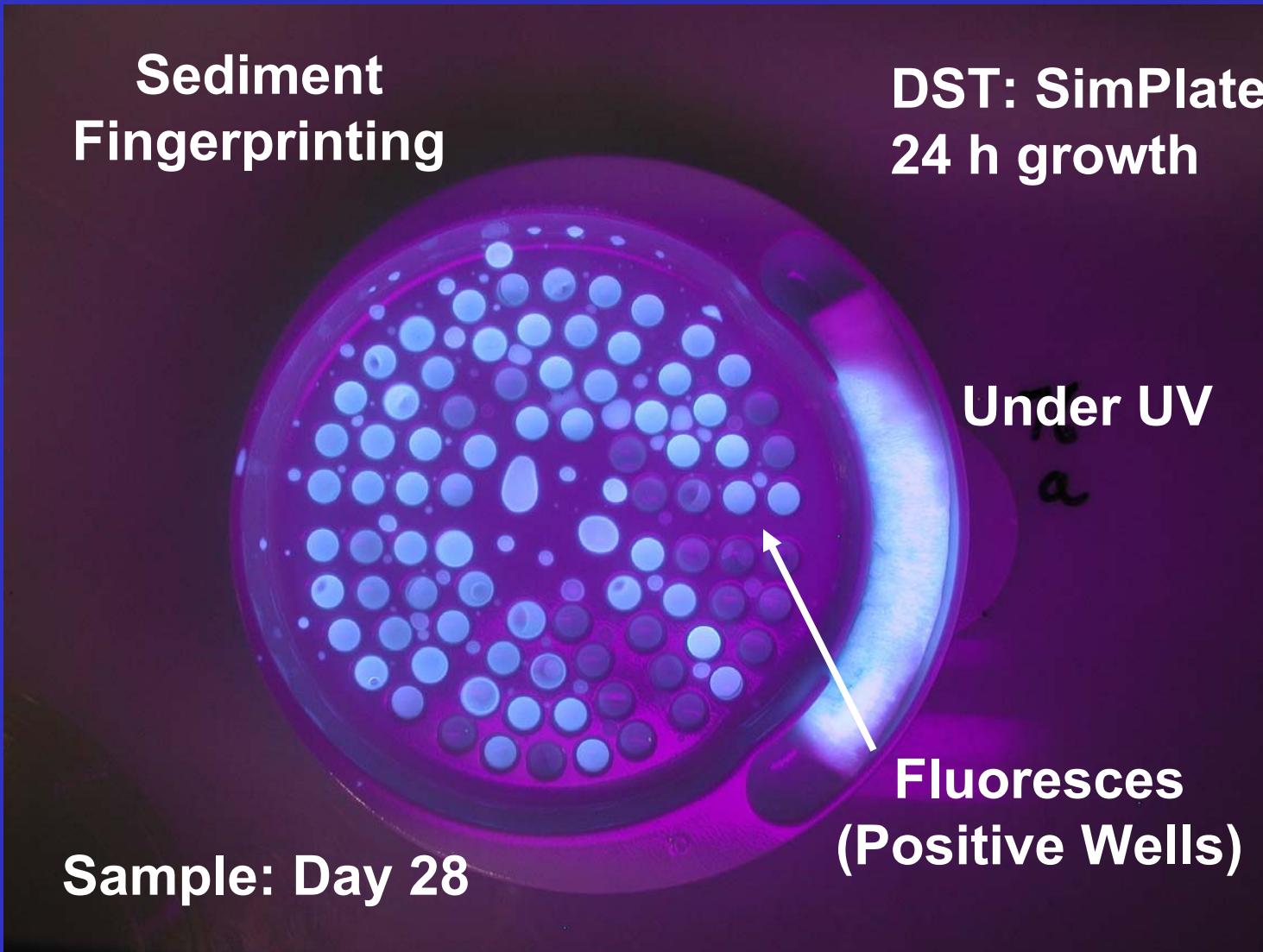
Sediment  
Fingerprinting

DST: SimPlate

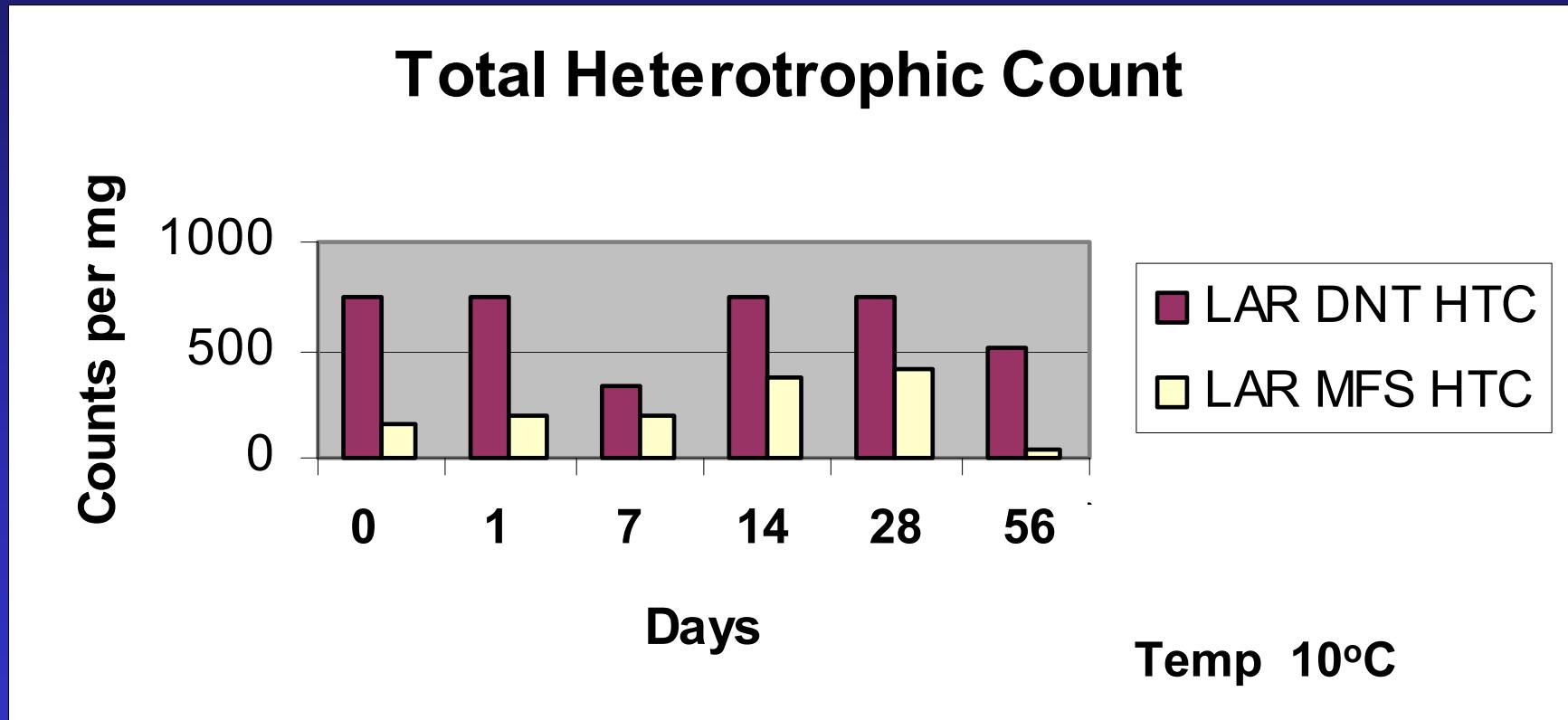


Wells

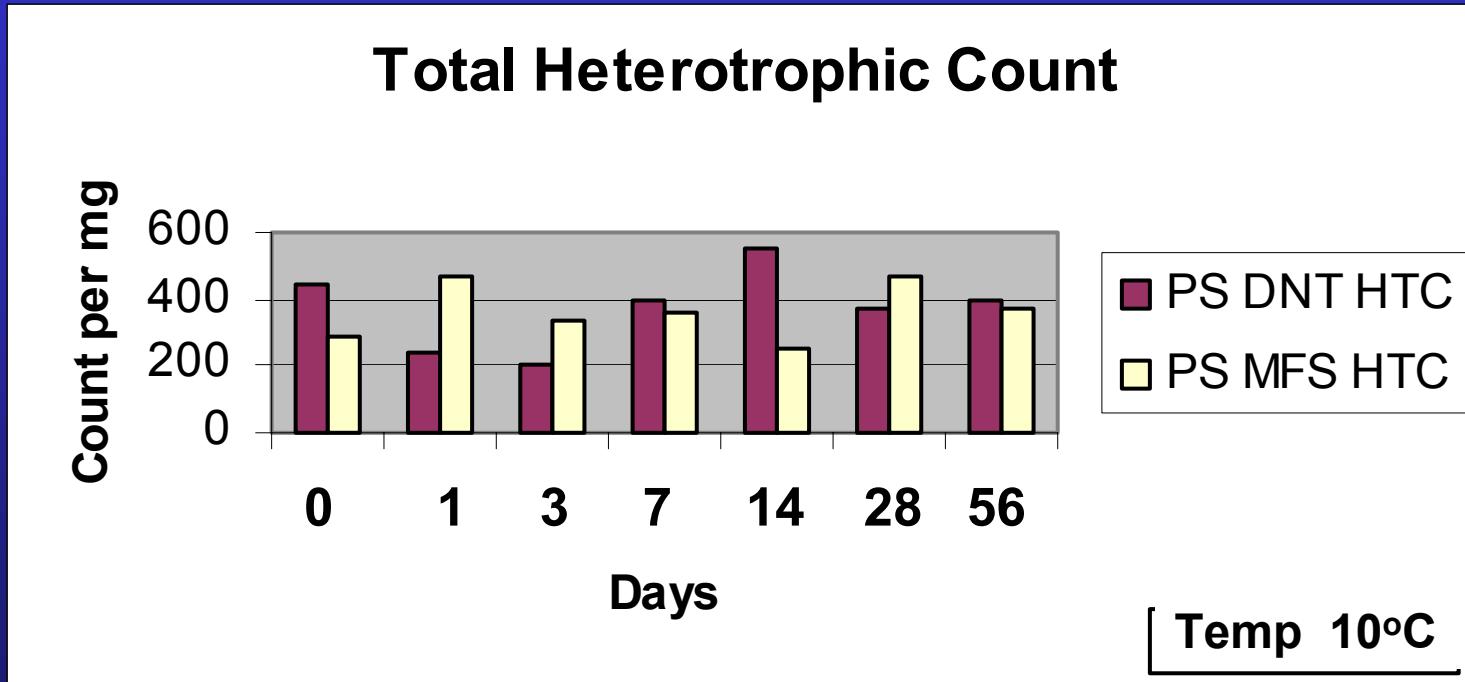
# Munitions Degradation Study: Puget Sound Sediment Analysis: Total Heterotroph Bacterial Count



# Heterotrophic bacterial perturbations during the degradation of dinitrotoluene (DNT) in Texas sediment.



# Heterotrophic bacterial perturbations during the degradation of dinitrotoluene (DNT) in Puget Sound sediments.



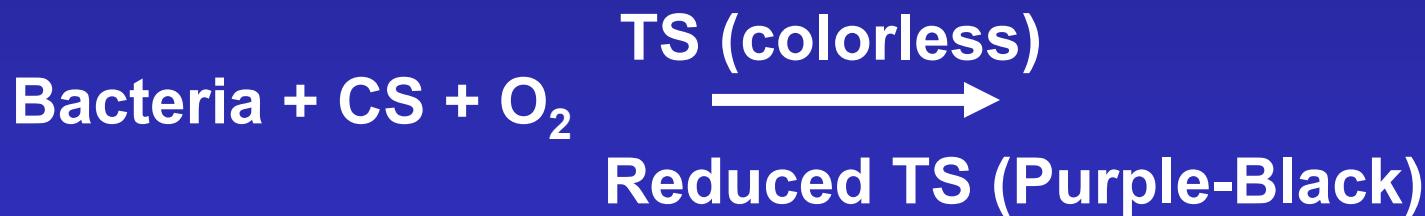
Counts per mg sediment wet weight; Simplate (Idexx)

# EcoPlate (Biolog) Microbial Community Analysis

## The well

Bacteria + Carbon Source (CS) + Indicator  
[Redox Tetrazolium salt (TS)]

The reaction:



$\text{CO}_2 + \text{H}_2\text{O} + \text{C fragments}$

Indicator: Purple-Black residue = positive reaction  
Substrate utilized by bacteria

# C-N Substrate in Ecoplates

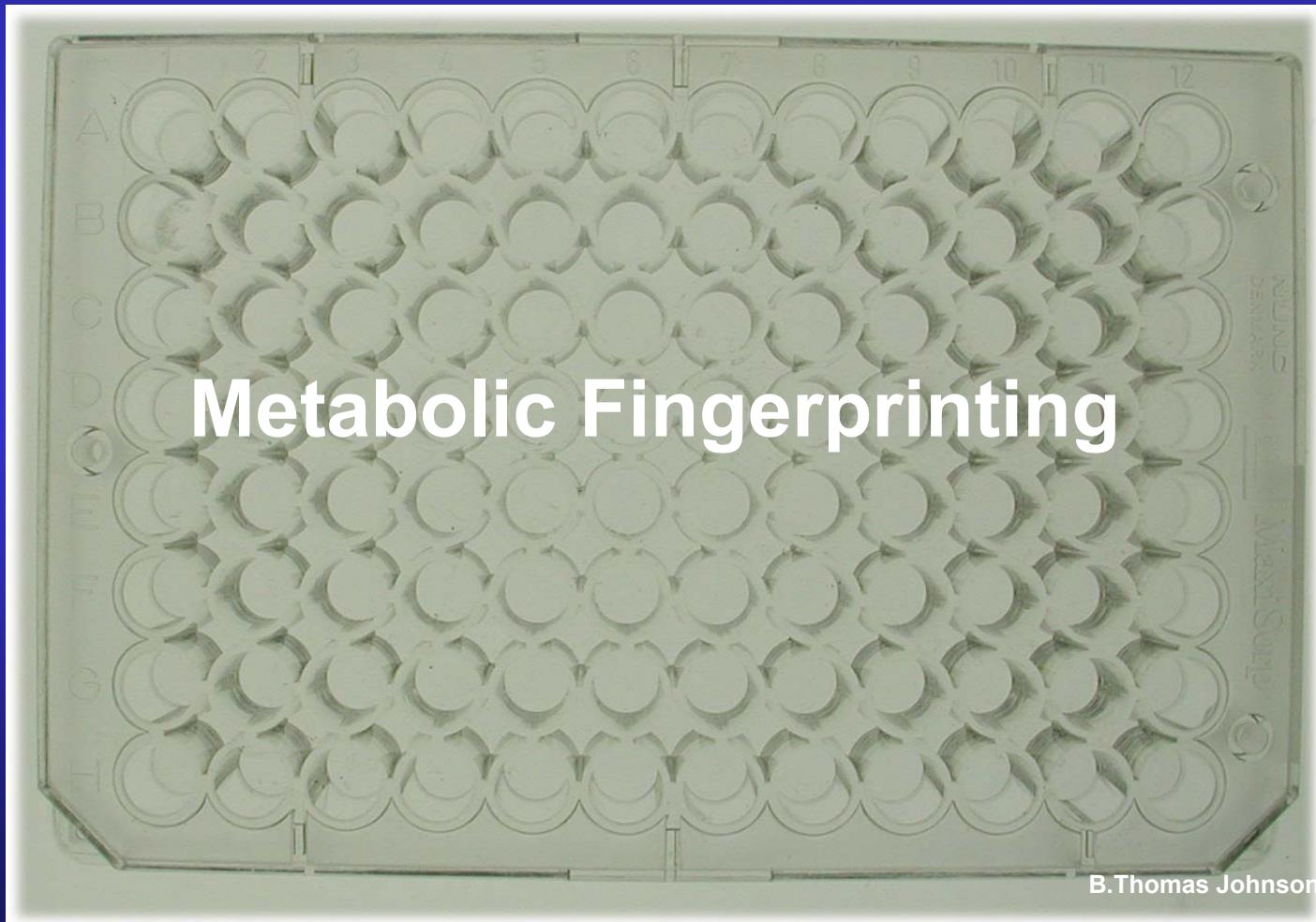
- Polymers
  - Glycogen
- Carbohydrate
  - $\alpha$ -d-Lactose
- Carboxylic Acids
  - D-galacturonic acid
- Amino acids
  - L-arginine
- Amines
  - Putrescine
- Phenolic compounds
  - 2-hydroxylbenzoic acid

# EcoPlate (Biolog) Microbial Community Analysis

## Blank Plate (96 wells)

1 2 3 4 5 6 7 8 9 10 11 12

A  
B  
C  
D  
E  
F  
G  
H



# EcoPlate (Biolog) Microbial Community Analysis

	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
<b>A</b>	Water	$\beta$ -Methyl Glucoside	D-Galactonic acid $\lambda$ lactone	L-Arginine
<b>B</b>	Pyruvic acid methyl ester	D-xylose	D-galacturonic acid	L-Asparagine
<b>C</b>	Tween 40	L-erythritol	2-hydroxy benzoic acid	L-Phenyl-alanine
<b>D</b>	Tween 80	D-Mannitol	4-hydroxy benzoic acid	L-Serine
<b>E</b>	$\alpha$ -Cyclodextrin	N-acetyl-D-Glucoamine	$\lambda$ -Hydroxybutyric acid	L-Threonine
<b>F</b>	Glycogen	D-Gluco-amine acid	Itaconic Acid	Glycyl-L-Glutamic Acid
<b>G</b>	D-Cellobiose	Glucose-1-PO <sub>4</sub>	$\alpha$ -Ketobutyric acid	Phenylethyl-amine
<b>H</b>	$\alpha$ -D-Lactose	D,L, $\alpha$ -Glycer-ol PO <sub>4</sub>	D-Malic Acid	Putrescine

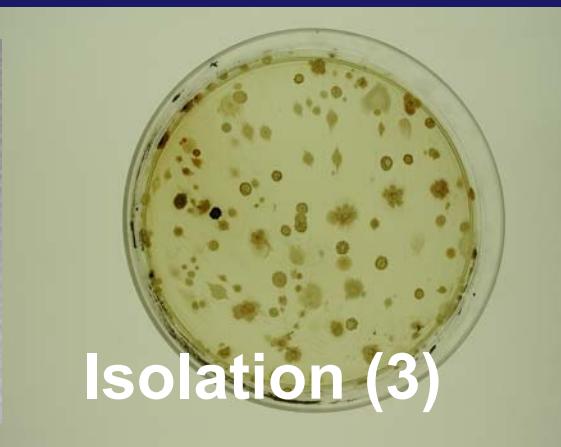
# Fingerprinting Protocol for Sediment Biodiversity



Selection (1)



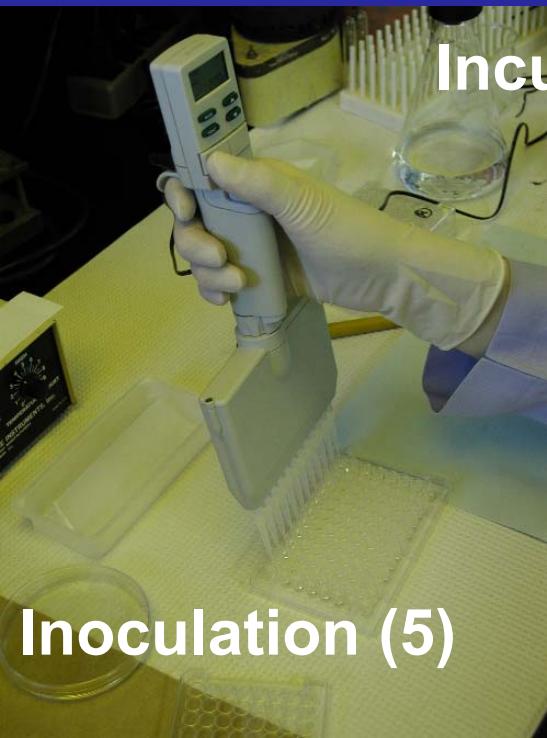
Growth (2)



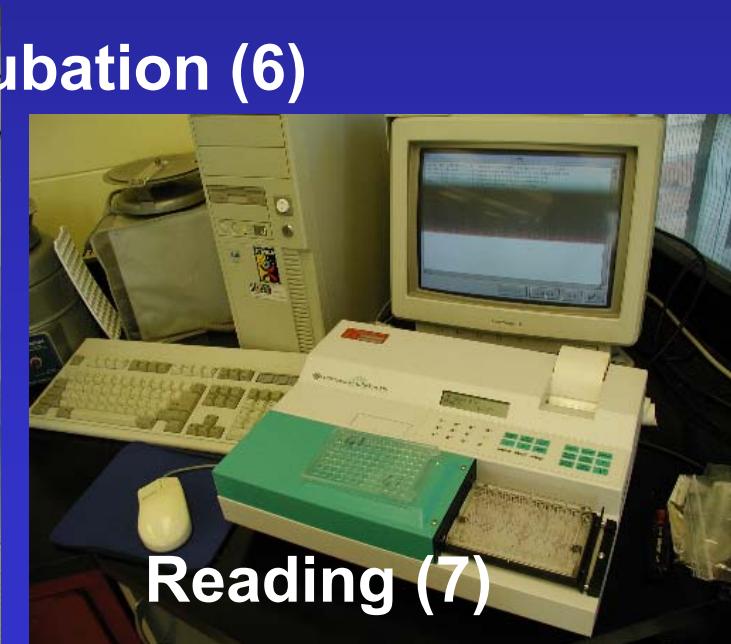
Isolation (3)



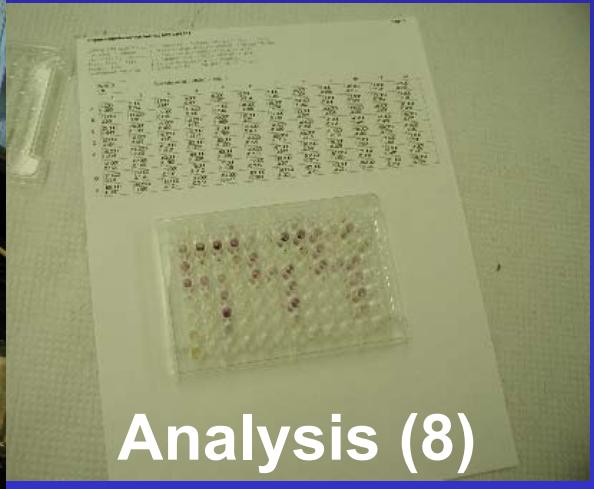
Suspension (4)



Inoculation (5)



Reading (7)



Analysis (8)

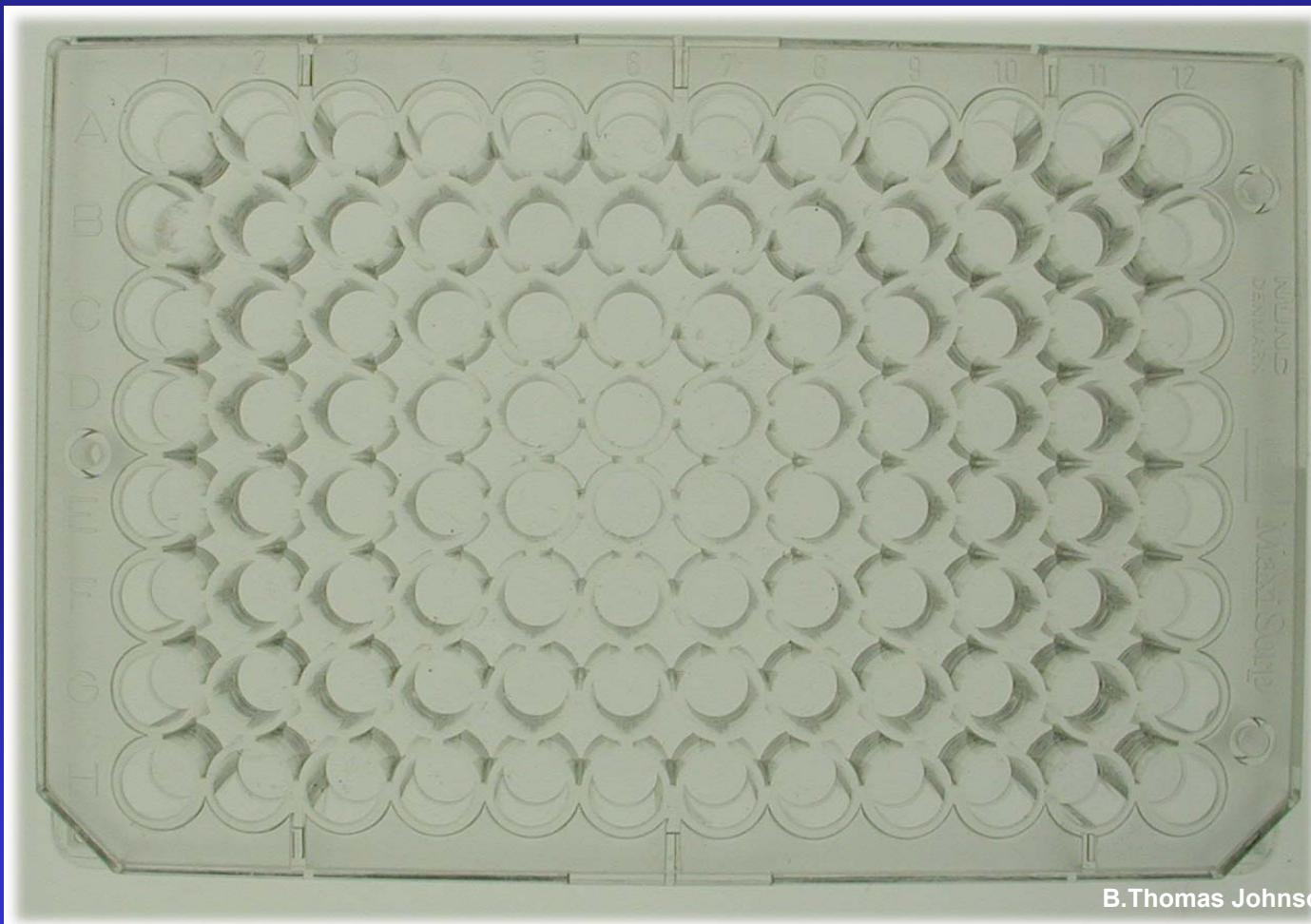
B.Thomas Johnson, CERC-USGS

# Metabolic Fingerprinting

Blank Plate (96 wells)

1 2 3 4 5 6 7 8 9 10 11 12

A  
B  
C  
D  
E  
F  
G  
H



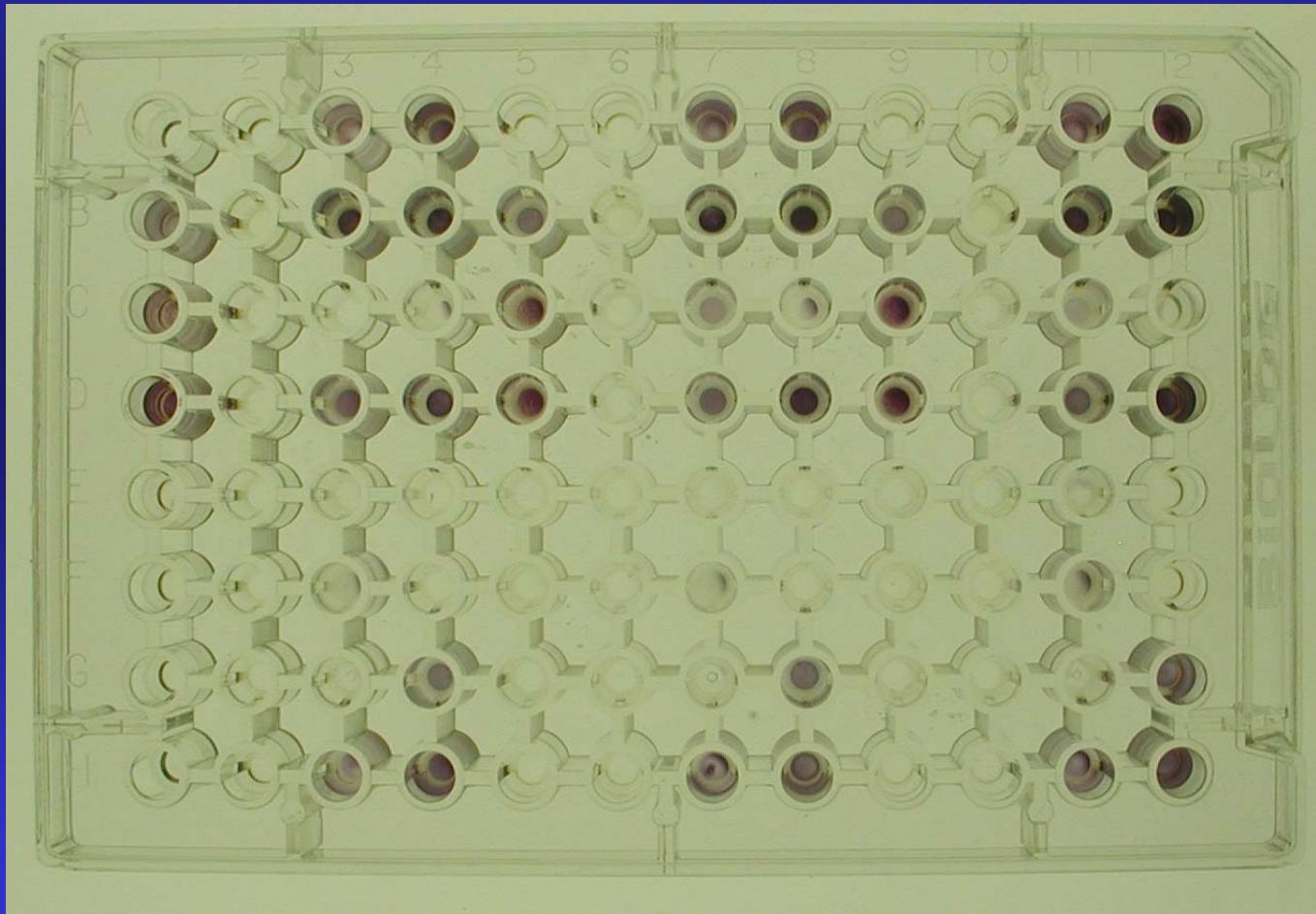
B.Thomas Johnson, CERC-USGS

# Metabolic Fingerprinting

CERC Pond Sediment

1 2 3 4 5 6 7 8 9 10 11 12

A  
B  
C  
D  
E  
F  
G  
H



# EcoPlate (Biolog) Microbial Community Analysis

CERC Pond Sediment

	1	2	3	4
A	Water	$\beta$ -Methyl Glucoside	Galactonic acid $\lambda$ lactone	Arginine
B	Pyruvic acid methyl ester	D-xylose	galacturonic acid	Asparagine
C	Tween 40	L-erythritol	2-hydroxy benzoic acid	L-Phenyl-alanine
D	Tween 80	D-Mannitol	4-hydroxy benzoic acid	L-Serine
E	$\alpha$ -Cyclodextrin	N-acetyl-D-Glucoamine	$\lambda$ -Hydroxybutyric acid	L-Threonine
F	Glycogen	D-Gluco-amine acid	Itaconic Acid	Glycyl-L-Glutamic Acid
G	D-Cellobiose	Glucose-1-PO <sub>4</sub>	$\alpha$ -Ketobutyric acid	Phenylethyl
H	$\alpha$ -D-Lactose	D,L, $\alpha$ -Glycer-ol PO <sub>4</sub>	D-Malic Acid	Putrescine

# Texas Control (LAR) Sediment: Metabolic Fingerprinting

## Day 1

Control

Hits: 13

	1	2	3	4
A				
B				
C				
D				
E				
F				
G				
H				

DNT

Hits: 7

Similar: 7

	1	2	3	4
A				
B				
C				
D				
E				
F				
G				
H				

Picric Acid

Hits: 11

Similar: 10

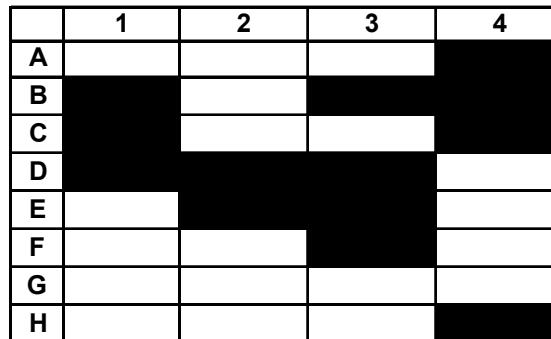
	1	2	3	4
A				
B				
C				
D				
E				
F				
G				
H				

# Texas Control (LAR) Sediment: Metabolic Fingerprinting

Day 7

Control

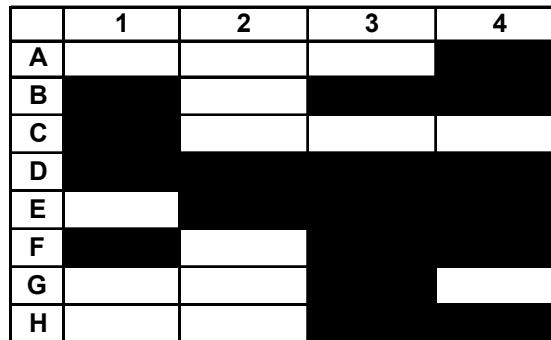
Hits: 13



DNT

Hits: 18

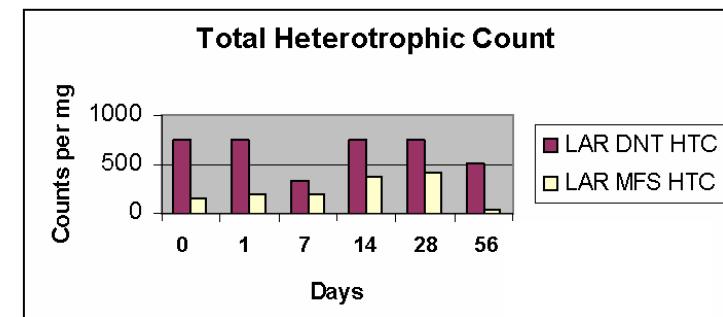
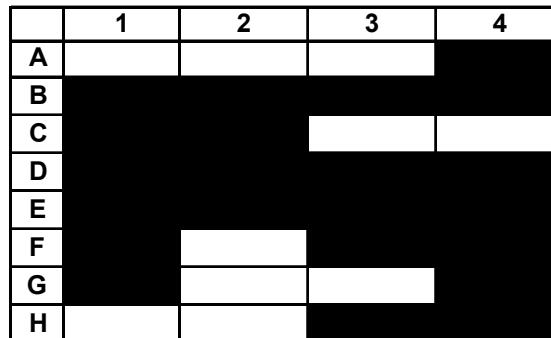
Similar: 11



Picric Acid

Hits: 22

Similar: 12



# Texas Control (LAR) Sediment: Metabolic Fingerprinting

Day 14

Control

Hits: 3

	1	2	3	4
A				
B				
C				
D				
E				
F				
G				
H				

DNT

Hits: 19

Similar: 3

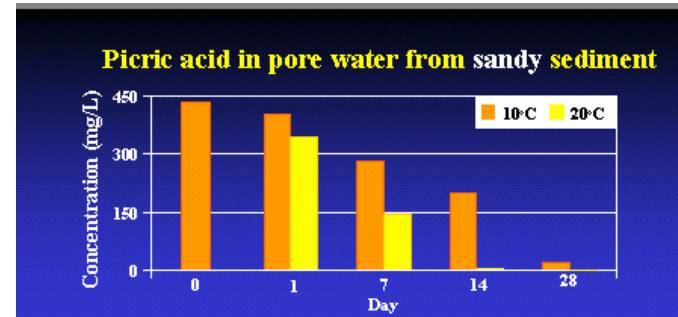
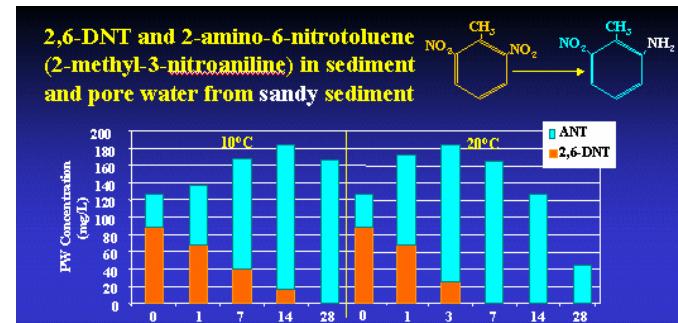
	1	2	3	4
A				
B				
C				
D				
E				
F				
G				
H				

Picric Acid

Hits: 22

Similar: 3

	1	2	3	4
A				
B				
C				
D				
E				
F				
G				
H				

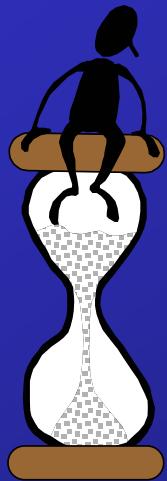


# Summary

## Uses of Indicator Bacteria in Aquatic Risk Assessment

- Eutrophication
- Toxicity
- Perturbation
- Biodiversity

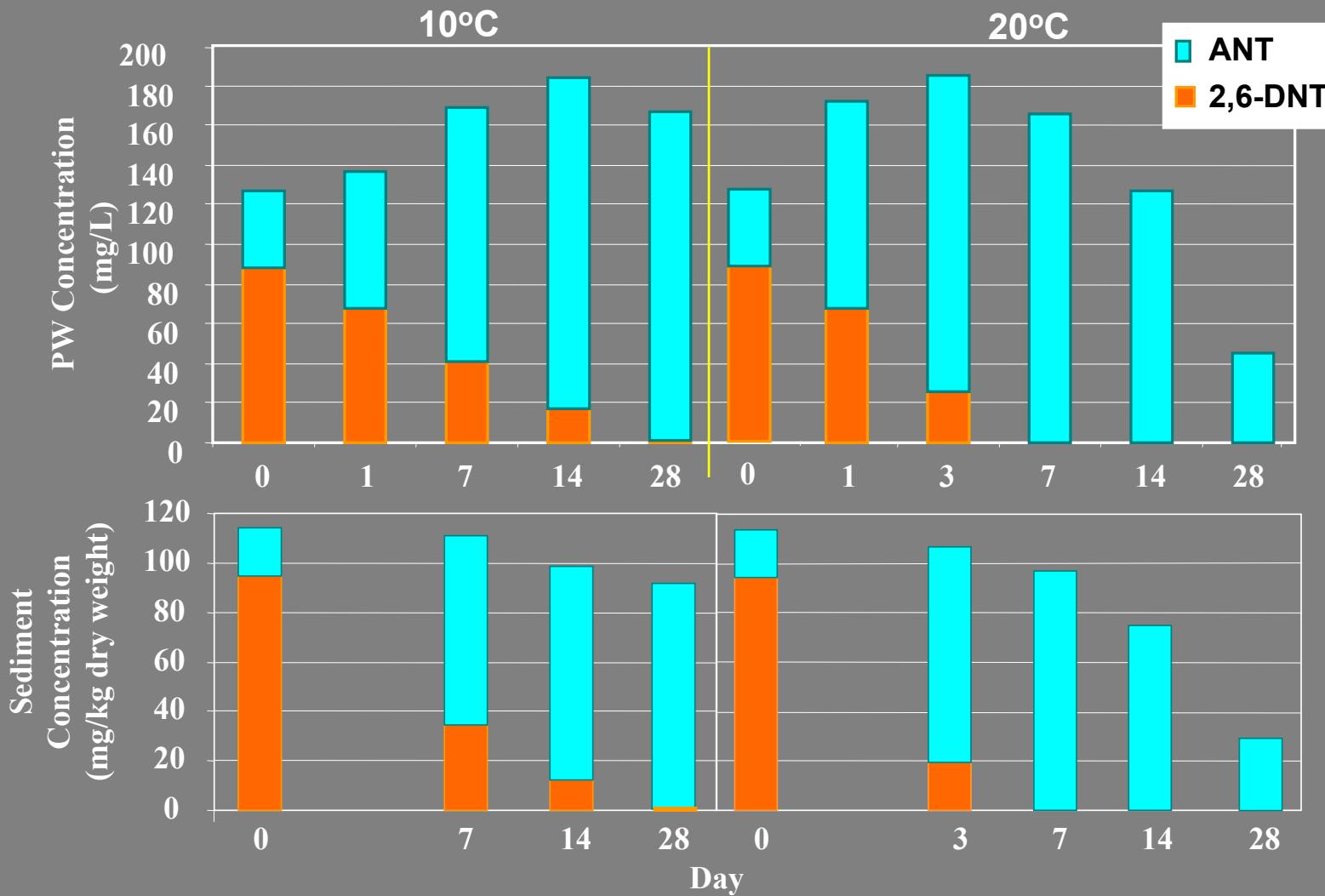
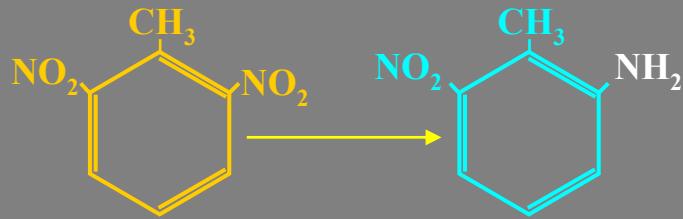
# Finis





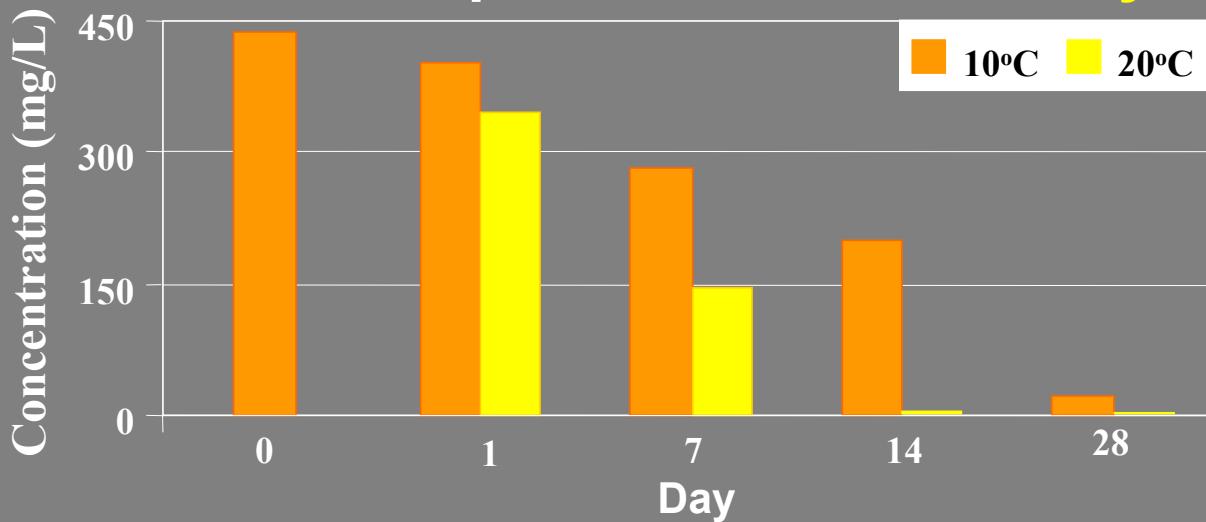
# Questions ???

# 2,6-DNT and 2-amino-6-nitrotoluene (2-methyl-3-nitroaniline) in sediment and pore water from sandy sediment



# Sediment Degradation

## Picric acid in pore water from sandy sediment



## Picric acid in pore water from muddy sediment

